Current Biology, Vol. 13, R778–R780, September 30, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.cub.2003.09.022

# Brain Mapping: New Wave Optical Imaging

Dispatch

**Thomas Mrsic-Flogel, Mark Hübener and Tobias Bonhoeffer**

**Optical imaging of intrinsic signals is widely used for high-resolution brain mapping in various animal species. A new approach using continuous data acquisition and Fourier decomposition of the signal allows for much faster mapping, opening up the possibility of applying this method to new experimental questions.**

Over the last two decades a number of imaging techniques have been developed, which allow for the visualization of brain regions activated by sensory stimuli. For human brain mapping, functional magnetic resonance imaging (fMRI) has proven to be particularly useful, since it is non-invasive and yields a spatial resolution of about 1 mm, which is often sufficient to assign activity to a specific area of the cortex. Even finer mapping of the brain is possible with optical imaging of intrinsic signals [1,2], which is based on small changes in light reflectance of neuronal tissue after its activation.

In principle, the technique of optical imaging is simple: the brain of an animal is exposed and illuminated with light of an appropriate wavelength (typically between 600 and 730 nm). Images are then acquired with a CCD-camera while the animal's sensory inputs are stimulated. Different components contribute to the overall intrinsic optical signal: in addition to activity-related changes in blood oxygenation level and blood volume, which are also measured in fMRI experiments, the light scattering properties of activated brain regions are altered. Upon neural activation, the absorption due to these different components increases, which in turn means that less incident light is reflected.

Because the reflectance changes are very small (typically 0.1% or less) in comparison to the overall uneven illumination of the brain surface, they are not directly visible. In order to visualize activated brain regions, images taken under different stimulation conditions have to be compared. The straightforward way to do this is to subtract images of the non-stimulated brain from those obtained when a stimulus was present ('blank-correction'). For example, in order to map the retinotopic organization of the mouse visual cortex [3], a small visual stimulus is presented at one location in the visual field and images are acquired over several seconds (Figure 1A). After an inter-stimulus interval of a few seconds, during which the intrinsic signal returns to baseline, the stimulus is presented at a different location and images are acquired, and so forth. Interleaved with these stimulus trials, images are taken in the absence of any visual stimulus, and these are used for blank-correction (Figure 1B). To improve the signal-to-noise ratio, each stimulus is presented between 8 and 32 times, and the resulting images are averaged. One thus obtains a set of activity maps, each displaying, as a dark patch, the cortical region activated by a particular stimulus position (Figure 1C). Typically, acquisition of such a set of maps takes between one and two hours. Further processing of these data can be used to generate maps that depict the overall retinotopic organization by assigning a color to each pixel, depending on which stimulus activated it best (Figure 1D).

An alternative paradigm for mapping cortical responses to different stimuli has been pioneered for fMRI [4,5]. It uses temporally periodic stimulus presentations and continuous image acquisition, and subsequent image processing in the time domain by Fourier analysis. This approach has been recently adapted for optical imaging by Kalatsky and Stryker [6] to map the functional organization of the visual cortex. When mapping cortical retinotopy in the mouse, the stimulus is a thin bar of light recurrently sweeping across the visual field at a fixed frequency (Figure 2A), during which a continuous image stream is recorded (Figure 2B). As the bar drifts, it sequentially activates neurons at neighboring cortical locations, resulting in a wave of activity across the visual cortex along one axis of the retinotopic map. Because of the repeated presentation of the moving bar, a cortical location is activated by its optimal stimulus position at precisely the same time, or the same phase, in the stimulus cycle. Again, however, as the intrinsic signal is so small, the raw image series does not reveal any meaningful patterns of cortical activity (Figure 2B) and further processing is necessary to obtain maps of retinotopy.

Consider the time-course of light reflectance at one pixel from a series of images of the mouse visual cortex (Figure 2C) recorded while the animal is viewing multiple repetitions of the mapping stimulus. The response is noisy and contains an oscillatory component on top of a slowly varying baseline. Separation of the unwanted noise components — caused by breathing, heart-beat and other sources — from the periodic stimulus-induced response can be achieved by Fourier analysis, which decomposes complex signals into component sinusoid functions of different frequencies. The power spectrum resulting from Fourier decomposition of the reflectance signal (Figure 2D) shows a prominent peak at ~0.17 Hz — the frequency at which the light bar was swept across the visual field. The time-course of the extracted Fourier component is shown in Figure 2E. When this procedure is applied independently at every pixel, the resulting image series reveals a pattern of cortical activity that shifts systematically with the position of the stimulus (Figure 2F).

The complete retinotopic map can then be computed by extracting, for each pixel, the phase and amplitude

Max-Planck-Institut für Neurobiologie, Am Klopferspitz 18A, D-82152 Martinsried, Germany. E-mail: flogel@neuro.mpg.de; mark@neuro.mpg.de; tobias.bonhoeffer@neuro.mpg.de

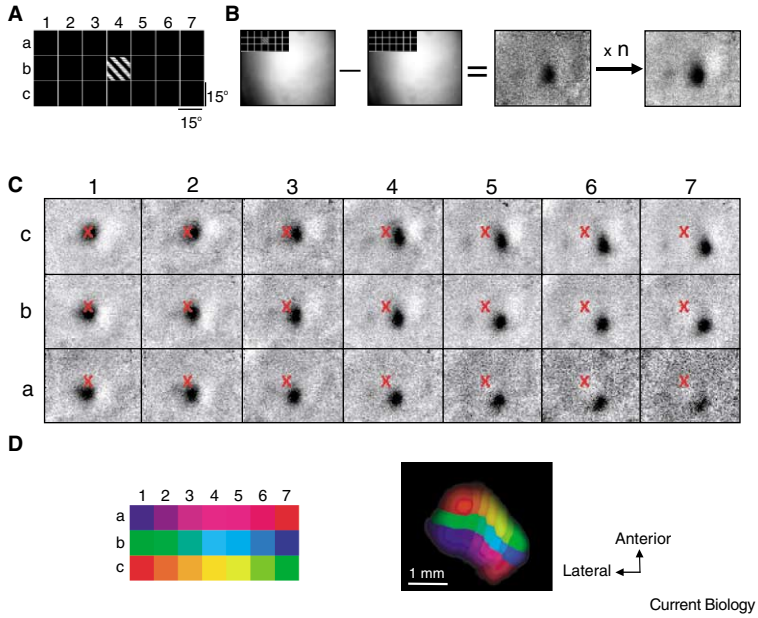


Figure 1. Data acquisition and image processing in conventional optical imaging experiments.

(A) For mapping retinotopy in mouse visual cortex, small visual stimuli are presented in random order at different locations in the visual field. (B) During stimulus presentation, reflectance images are obtained from the cortical surface. The raw images do not reveal a meaningful activation pattern. Subtracting images obtained in the absence of a stimulus from those acquired while a stimulus was shown reveals a dark patch, corresponding to the activated region in the cortex. Averaging over several stimulus presentations increases the signal-to-noise ratio. (C) An activity map is derived for each stimulus location. Note the systematic shift of the activated region relative to the red cross, which was placed at the same position in all maps. (D) A complete map of retinotopy is generated by assigning a color to each pixel, based on which stimulus location it responded to best. The response strength is encoded by pixel intensity, such that non-responsive regions of the cortex appear dark.

of the Fourier component at the stimulation frequency. The phase (Figure 2G) is directly related to the position of the bar in the stimulus cycle, while the amplitude indicates the strength of the response at that pixel. But because of the relatively long delay of the intrinsic signal (several hundred milliseconds), the raw phase map is only a representation of relative retinotopy, as it reveals the timing of the optical signal rather than that of the stimulus. Computing absolute retinotopy therefore requires subtracting from the phase map the delay in the cortical response (due to the slow time-course of the optical signal as well as neural response latencies), which should both be equal for every responsive pixel and have a constant phase value.

The delay (Figure 2I) is computed from addition of phase values of maps obtained with opposite-direction stimuli (Figure 2G,H). Whereas reversal of the stimulus direction reverses the cortical response sequence, the delay at each pixel should stay the same (Figure 2I). One thus obtains a continuous retinotopic map of one axis (elevation) of visual space (Figure 2J). Maps of the orthogonal orientation (azimuth) can be extracted by stimulation with bars of orthogonal orientation, thereby allowing two-dimensional reconstruction of visual space representation by the visual cortex.

Continuous stimulation and image acquisition combined with Fourier analysis offers several advantages over the conventional approach. First, global changes in light reflectance and hemodynamic noise are efficiently removed from the signal of interest, provided they do not occur at the stimulus frequency. Second, spatially continuous stimulation allows fine-grained sampling of the stimulus space, avoiding the need to interpolate to generate smooth retinotopic maps. Third, as periodic stimulation allows more stimulus repetitions in a given time than conventional imaging, good quality retinotopic maps can be acquired in as little as several minutes. Finally, time could be economized even further by testing two stimulus features at the same time but at different repetition frequencies. As an example, consider two bars, one sweeping through the visual field horizontally with a period of 6 seconds and another sweeping vertically with a period of, say, 5 seconds. The resulting maps could then easily be decomposed in the Fourier domain and the complete retinotopy can be mapped in one go. While for this example non-linear interactions of the stimuli might present a problem, other stimulus features that show less interaction could be mapped simultaneously with such an approach.

There are some limitations to this elegant and time-efficient technique. As in conventional optical imaging, the main, unavoidable constraint on mapping resolution is the large spatial spread of the measured response. This is a consequence of neural circuitry [3,7] and the finite spatial resolution of intrinsic signals, which together cause a single point in visual space to induce reflectance changes in a rather large cortical area. The new imaging paradigm also cannot circumvent this constraint and does thus not provide a much more precise mapping of stimulus space. It is important to distinguish between the precision with which the map can be calculated — indeed higher with the new approach — the resolution that intrinsic signal optical imaging provides — still a matter of debate and highly dependent on wavelength, physiological state of the animal, and so on — and the cortical point spread function (the precision with which a point in space is represented on the cortical surface). The latter two are fundamental limitations to the precision with which the map of visual space can be determined with any optical imaging approach. In this sense, the 40-fold improvement of resolution stated in the paper is slightly misleading since it is theoretically correct, but not really achievable under the biological circumstances. A better way to test the 'true' resolution of the new technique is to determine how large a gap has to

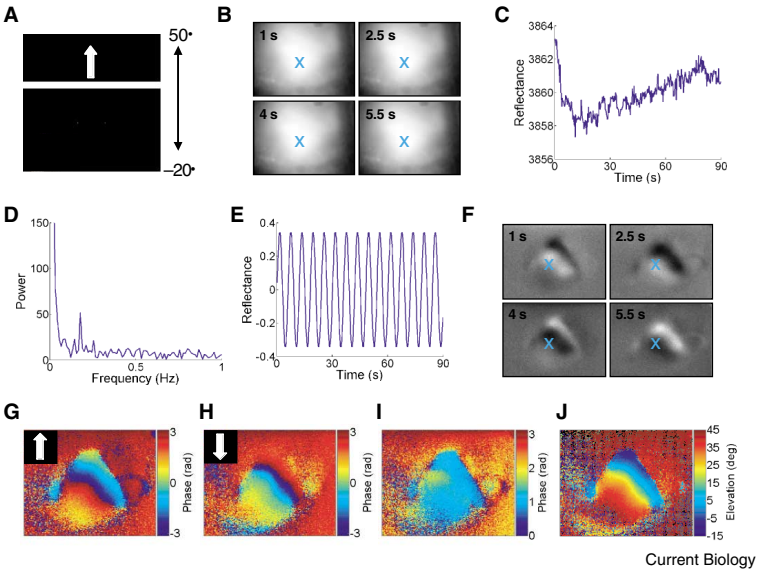


Figure 2. Data acquisition and image processing of the new optical imaging paradigm.

(A) The mapping stimulus is a thin bar of light that sweeps across the visual field at a fixed frequency (here, the period is 6 sec). (B) Raw images taken at different times during one stimulus cycle (as indicated). (C) Time-course of the reflectance signal at the pixel demarcated by the blue cross in (B) over 15 presentations of the stimulus. (D) Fourier analysis (power spectrum) of the signal in (C), showing the relative contributions of different frequency components. Note the prominent peak at ~0.17 Hz, the same frequency as the stimulus. Here, power is equivalent to signal amplitude. (E) Restored Fourier component at 0.17 Hz, after removing all other frequency components. (F) When this filtering sequence in (D,E) is applied to each pixel independently, the restored images reveal a dark stripe across the cortex, which changes its position depending on the time in the stimulus cycles. (G) Extracted phase values from the 0.17 Hz Fourier component at each pixel in the image show a smooth progression of phase across the cortex. (H) Phase map from the reverse direction of the stimulus (downward drift). Conversion of phase (radians) into stimulus position (degrees) requires subtraction of the cortical response delay (I) from the phase values. The delay is computed by halving the sum of upward and downward phase values. (J) Absolute map of retinotopy in degrees of visual angle, revealing the extent of the left primary visual cortex.

be introduced into the mapping stimulus until it can be reliably detected in the map.

There are also potential sources of error in mapping accuracy. These may be small but they are not negligible and need to be considered. One source derives from the fact that, depending on the stimulus used, the response delays may not be equal for all parts of the cortex (Figure 2I). In a retinotopic map, for example, pixels at map 'edges' may have different latencies depending on stimulus direction, that is, whether they were activated at the beginning or end of the sweep period. At sweep onset, a pixel's response directly reflects the presence of the stimulus, whereas toward the end of the sweep, the response is likely a compound result of the actual stimulus-induced response, the forward-advancing wave of cortical activity through intracortical connections, and local hemodynamic influences which may change with stimulus procession. The resulting response-delay differences at map extremities to opposite stimulus directions will systematically confound the map accuracy because the computation of absolute retinotopy assumes fixed delay values. An additional concern is related to the fact that with the Fourier approach the time-course of the signal at each pixel is necessarily represented by a single sinusoid (from which phase is calculated). As the original signal often is very different from a sinusoid, this can introduce small but systematic artifacts in the maps of absolute retinotopy. Analysis of signals by stimulus-triggered averaging or wavelet decomposition [8] can partly remedy this problem. Finally, it should also be noted that in the new approach the timing of the stimulus repeat rate is adjusted well to known temporal properties of the intrinsic signal itself, such as response dynamics and frequencies of noise components. Most likely much faster (conceivably also slower) repetition rates will work less well. This entails that testing the mapping of temporal properties of the stimulus can be less straightforward with the new approach.

These examples show that this new technique has its caveats and limitations. Nevertheless, the extraction of activation patterns by Fourier decomposition is a highly welcome addition to the arsenal of optical imaging acquisition and analysis tools. It will prove useful for many applications in particular under conditions where the overall data acquisition time is limited. This is true for chronic experiments, in which anaesthesia duration should be minimised, as well as for experiments in awake animals. In particular, intraoperative imaging in human patients should greatly benefit from the substantial reduction in imaging time.

## References

1. Grinvald, A., Lieke, E.E., Frostig, R.D., Gilbert, C.D. and Wiesel, T.N. (1986). Functional architecture of cortex revealed by optical imaging of intrinsic signals. Nature *324*, 361-364.
2. Bonhoeffer, T. and Grinvald, A. (1996). Optical imaging based on intrinsic signals: The Methodology. In Brain Mapping: The Methods. Edited by Toga AW, Mazziotta JC. San Diego, CA: Academic Press, Inc.; 55-97.
3. Schuett, S., Bonhoeffer, T. and Hübener, M. (2002). Mapping retinotopic structure in mouse visual cortex with optical imaging. J. Neurosci. *22*, 6549-6559.
4. Engel, S.A., Rumelhart, D.E., Wandell, B.A., Lee, A.T., Glover, G.H., Chichilnisky, E.-J. and Shadlen, M.N. (1994). fMRI of human visual cortex. Nature *369*, 525.
5. Sereno, M.I., Dale, A.M., Reppas, J.B., Kwong, K.K., Belliveau, J.W., Brady, T.J., Rosen, B.R. and Tootell, R.B.H. (1995). Borders of multiple visual areas in humans revealed by functional magnetic resonance imaging. Science *268*, 889-893.
6. Kalatsky, V.A. and Stryker, M.P. (2003). New paradigm for optical imaging: Temporally encoded maps of intrinsic signal. Neuron *38*, 529-545.
7. Das, A. and Gilbert, C.D. (1995). Long-range horizontal connections and their role in cortical reorganization revealed by optical recording of cat primary visual cortex. Nature *375*, 780-784.
8. Laine, A.F. (2000). Wavelets in temporal and spatial processing of biomedical images. Annu. Rev. Biomed. Eng. *2*, 511-550.